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EXAMINER	
MARVICH, MARIA	
ART UNIT	PAPER NUMBER
1633	

SHORTENED STATUTORY PERIOD OF RESPONSE	MAIL DATE	DELIVERY MODE
3 MONTHS	03/12/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

If NO period for reply is specified above, the maximum statutory period will apply and will expire 6 MONTHS from the mailing date of this communication.

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Office Action Summary	Application No.	Applicant(s)	
	10/774,122	ZWAKA ET AL.	
	Examiner	Art Unit	
	Maria B. Marvich, PhD	1633	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *04 January 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-16* is/are pending in the application.

4a) Of the above claim(s) *5,6,11,14-16* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4, 7-10, 12 and 13* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *10/12/04 10/21/05*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

Applicants' election with traverse of Group I in the reply filed on 1/4/07 is acknowledged. The traversal is on the ground(s) that the subject matter of Groups I and II is linked because they overlap in scope and a search for art for one would identify art relevant to the other. Furthermore, applicants argue that restriction is optional.

The arguments filed 1/4/07 have been considered but are not persuasive for the following reasons. Group I and Group II are related as the method of Group I is required to make the product of Group II. However, as set forth in the office action mailed 10/6/06, the inventions have been determined to be distinct. According to US restriction practice, inventions may be related and still be patentably distinct. See MPEP 802.01 and 806.05(h), "The term "distinct" means that two or more subjects as disclosed are related, for example, as combination and part (subcombination) thereof, process and apparatus for its practice, process and product made, etc., but are capable of separate manufacture, use, or sale as claimed, AND ARE PATENTABLE (novel and unobvious) OVER EACH OTHER (though they may each be unpatentable because of the prior art). It will be noted that in this definition the term related is used as an alternative for dependent in referring to subjects other than independent subjects." In the instant case, the products can be made by a materially different process and hence a search of any given process does not adequately support patentability of the product. Conversely, a determination of whether the method of making the product is patentable over the art is based upon the particulars of the method and not on the product made by or used in the method. Hence, a search for art for either the method or the product does not necessarily evince the other.

The MPEP teaches that (803) "the search and examination of all the claims in an application can be made without serious burden, the examiner must examine them on the merits, even though they include claims to independent or distinct inventions." As guidance, the MPEP teaches "For purposes of the initial requirement, a serious burden on the examiner may be prima facie shown if the examiner shows by appropriate explanation of separate classification, or separate status in the art, or a different field of search as defined in MPEP § 808.02. That prima facie showing may be rebutted by appropriate showings or evidence by the applicant" (see MPEP 803). In the instant case, a search burden has been demonstrated . Briefly, the restriction requirement states that Group I and II have a separate status in the art, and require different fields of search and demonstrate that the Groups have distinct classification. Given the search burden, the claims have been appropriately restricted. The requirement is still deemed proper and is therefore made FINAL.

Claims 5, 6, 11 and 14-16 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected subject matter, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 1/4/07.

Claim Objections

Claim 7 is objected to because of the following informalities: in claim 7 the recitation "identifying cells expressing the marker gene in the genetic construct". However, the marker is not expressed "in the genetic construct" but --from the genetic construct--. It would be remedial to amend the claims to reflect this.

Claim 8 is objected to because of the following informalities: claim 8 and 7 refer to the same cells in alternative means i.e. claim 7 refers to "cells of a defined lineage" while claim 8 uses the term "cells of the desired lineage". It would be remedial to use the same term throughout the claims for consistency. Secondly, claim 8 uses a promoter "which is active to express a gene only in cells", which would be preferably described as --which is active only in cells--.

Claim 10 is objected to because of the following informality: claim 10 recites "the marker gene expresses" whereas the gene does not "express" a protein but --encodes-- it.

Appropriate correction is required.

Claim Rejections - 35 USC § 112, second paragraph

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter, which the applicant regards as his invention.

Claims 1, 7-10, 12 and 13 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being incomplete for omitting essential steps, such omission amounting to a gap between the steps. See MPEP § 2172.01. The omitted steps are: claim 1 lacks a step that allows identification of cells in the absence of marker. Without a marker it is not clear how the cells comprising the genetic construct will be identified.

Claim 7 is vague and indefinite in that the metes and bounds of "stem cell in culture" are unclear. The preamble recites that there are "cultures of human embryonic stem cells" whereas only one culture is referenced in line 8. It is not clear if all the cells are subjected to purification or just one of the cultures by recitation of stem cell in culture. As well, it would be remedial to reference the cell using an article for proper antecedent basis. If it is all of the cultures of stem cells it would be remedial to recite --the cultures of human embryonic stem cells-- or -- the human embryonic stem cells in culture--.

Claim 7, line 10 is vague and indefinite in that the metes and balances of "purifying those cells from other cells" are unclear. The method involves electroporation of a construct into ES cells in which cells expressing marker are identified followed by "purifying those cells from other cells". While it appears that "those cells" refer to the cells expressing marker, it would be more clear to reference the cells directly as --cells expressing marker--. However, it is not clear what the "other cells" reference and where these cells originate. It would be remedial to identify the "other cells" as other is a relative term for which the claims do not provide the requisite metes and bounds.

Claim 12 recites the limitation "the method of claim 6" in claim 12. There is insufficient antecedent basis for this limitation in the claim. Claim 6 is drawn to a cell, which is drawn to non-elected subject matter. If as it appears, the recitation in claim 12 should be "the method of claim 7", it would be remedial to amend the claims accordingly. For purposes of art, it will be understood that claim 12 depends from claim 7 and not claim 6.

Claim 12 is vague and indefinite in that the metes and bounds of the term "derived from" are unclear. It is unclear the nature and number of steps required to obtained a "derivative" of

hES cells. The term implies a number of different steps that may or may not result in a change in the functional characteristics of the defined lineage from the source that it is "derived from". It would be remedial to recite that the cells of a defined lineage are --obtained-- from the hES.

Claim 12 is vague and indefinite in that the metes and bounds of " culturing non-transformed ES cells so that the ES cells initiate differentiation; and purifying the cells of the defined lineage based upon the expression of the genes identified in the analyzing step" are unclear. First, it is not clear to what "the ES cells" in the phrase above refer as there are cells of the defined lineage and non-transformed ES cells. Secondly, the cells of the defined lineage were purified previously as stated in line 3. Hence, it is not clear from what the already purified cells are being purified. Thirdly, it is not clear how ES cells can "initiate" differentiation, which implies that the cells themselves are responsible for the "initiation" and as well the cells do not finish differentiation. Finally, it is not clear what role this step plays as the non-transformed cells that are cultured do not appear to be part of any other steps in the method. If the cells are meant to be the cells of the defined lineage in line 7 that are purified, it is not clear how in claim 13, the defined lineage can be undifferentiated cells then as differentiation has been initiated and hence the cells cannot be in a completely undifferentiated state.

Claim Rejections - 35 USC § 112, first paragraph

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-4, 7-10, 12 and 13 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for targeted modifications of embryonic stem cells by electroporating copies of 1) a targeting vector comprising a foreign gene and a marker flanked by 3' and 5' homologous arms and 2) identifying cells which contain the genetic construct by detection of the marker 3) in the absence of a promoter, the construct is designed to recombine such that the marker gene is operably linked to an endogenous, tissue specific promoter 4) and further for purifying cells of a defined lineage, the marker gene comprises a promoter active in cells of a defined lineage, the specification does not reasonably provide enablement for any other embodiment. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The test of enablement is whether one skilled in the art could make and use the claimed invention from the disclosures in the patent coupled with information known in the art without undue experimentation (*United States v. Telectronics, Inc.*, 8 USPQ2d 1217 (Fed. Cir. 1988)). Whether undue experimentation is required is not based on a single factor but is rather a conclusion reached by weighing many factors (See *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Inter, 1986) and In *re Wands*, 8USPQ2d 1400 (Fed. Cir. 1988); these factors include the following:

1) Nature of invention. The instant claims are drawn to a method of targeted modification of human embryonic stem cells by electroporation with a construct designed for homologous recombination. Cells transformed with the construct are identified and purified.

2) Scope of the invention. The scope of the invention is extremely broad in that the cells are modified by transformation with *any* foreign gene. While claims 2-4 recite that the construct includes the marker, it is not clear if the marker is used in identification or for some other purpose in the method. Without the marker, the method of identification would require screening the transformed cells for possession of the construct by hybridization given the broad nature of the construct used to transform the cells. In claim 3, the marker is inserted into *any* location such that an endogenous promoter expressed only in a desired state is operable linked to the marker. Alternatively in claim 4, the marker a tissue specific promoter functional only in "cells in a desired state of differentiation". Claim 7 is drawn to methods of purifying cells of *any* defined lineage from cells that have been transformed as above. In this method, the construct comprises a marker, however, it is only said broadly that the marker gene will be expressed only in cells of the defined lineage and the marker is used for identification and purification. Claim 8 limits the marker a tissue specific promoter functional only in active in cells of the desired lineage. The cells can be induced or permitted to differentiate following transformation. Claim 12 appears drawn to methods of identifying genes in the purified cells such that cells in that defined lineage can be purified based upon the expression of the identified genes.

3) Number of working examples and guidance. The present invention is drawn to methods of modifying hES cells by electroporation using constructs designed for homologous recombination. Inserted genes are for generation of "knock-ins" or "knock-outs" in which a gene

is either enhanced or disrupted respectively. Also the specification teaches methods of purification of cells based upon insertion of the genes into specific locations within the genome. However, only a single location is described in the specification from which the lineage of the cell can be assayed and this is the Oct4 region where a construct comprising a promoterless construct is inserted into the 3'UTR region. The region is only active during The specification teaches that the genetic construct should comprise two homologous arms that are identical to regions of the genome 3' and 5' of the location at which the genetic insert is to be inserted.

4) State of the art. The art of manipulation of non-human embryonic stem cells at the time of filing of the instant application was well-established. However, less so for human embryonic stem cells. However, it has been shown that "The pluripotency of the hES cells was established by demonstration of their ability to differentiate into all three germ layers. hES cells injected into immunodeficient mice formed benign teratomas containing advanced differentiated tissue types, representing all three germ layers.Another approach establishes ES pluripotency by *in vitro* differentiation. A variety of studies have described *in vitro* spontaneous and directed differentiation of hES cells to different lineages: cardiomyocytes,neurons and glia, endothelial cells, hematopoietic precursors, trophoblast, and hepatocyte-like cells. The most common method used for *in vitro* differentiation is to remove the hES cells from the feeder layer and culture in suspension in absence of mouse embryonic fibroblasts (MEFs). (Lev et al page 54, ¶ 3). If the ES cells are permitted to have significant contact with each other, they will spontaneously begin to aggregate into clumps and begin differentiation. Otherwise, the cells are driven to differentiation by addition of chemicals, growth factors, cytokines or hormones.

5) Unpredictability of the art. The MPEP teaches, "However, claims reading on significant numbers of inoperative embodiments would render claims non-enabled when the specification does not clearly identify the operative embodiments and undue experimentation is involved in determining those that are operative. Atlas Powder Co. v. E.I. duPont de Nemours & Co., 750 F.2d 1569, 1577, 224 USPQ 409, 414 (Fed. Cir. 1984); In re Cook, 439 F.2d 730, 735, 169 USPQ 298, 302 (CCPA 1971). (see MPEP 2164.08(b).

Applicants' claims are broadly drawn to a genetic construct that includes *any* foreign gene and "regions at either of its ends homologous with a pair of selected regions in the genome of the ES cells". Claims 1 and 7 recite "obtaining copies of a genetic construct which includes a foreign gene and which has regions at either of its ends homologous with a pair of selected regions in the genome" are unclear. It is highly unpredictable that such a vector allow for homologous recombination given the broad nature of the recited regions in the genome, which as such can be on distinct chromosomes or distinct parts of the chromosome. Rather the construct must be a targeting vector which comprises 5' and 3' arms flanking the insert that are homologous to a genomic region flanking a site designated for insertion. Secondly, the ability to identify cells comprising the genetic construct absent a marker is highly unpredictable and so the construct requires that the foreign gene also comprise a marker for cellular identification. Absent a marker, methods of selecting for the gene would appear to involve PCR, Southern analysis and means of selecting for growth of the cell based upon possession of the gene or reduced production of the gene product. For the later, the methods can rely on directly assaying for the gene product or if it does not have a reliable means of being assayed, the protein can be assayed by Western analysis. However, methods of screening based upon many of these

techniques based upon Southern or Western analysis or PCR are laborious, potentially futile and highly unpredictable. Scheid et al teach identification of targets "that have successfully incorporated transgenic DNA is somewhat like finding a transgenic needle in a non-transgenic haystack (see page 398, col 1, paragraph 1). Applicants' exemplified methods require use of markers.

Thirdly, the claims are drawn to insertion of the construct into *any location* in the genome wherein the a marker gene is expressed in cells in a desired state of differentiation. The only means of so doing as described by the art and the specification is a location comprising the regulatory region of a gene that is selectively expressed in the desired lineage or state of differentiation. Applicants post-filing publication for example teaches insertion of a promoterless marker construct into hES to identify undifferentiated cells. "To test this approach, we introduced two reporter genes into the Oct4-encoding gene *POU5F1* by homologous recombination. Oct4, which belongs to the POU (Pit, Oct, Unc) family of transcription factors, is expressed exclusively in the pluripotent cells of the embryo and is a central regulator of pluripotency. We introduced two promoterless reporter-selection cassettes into the 3' untranslated region (UTR) of *POU5F1*. (Zwaka and Thomson) The pluripotent state-specific transcription factors Oct4 was highly expressed in undifferentiated hES cells, and its expression gradually decreased during culturing of cells in suspension and after plating (Zwaka and Thomson, 2003, page 319, col 2, ¶ 3)." By any other means, the success of identifying means of using a marker gene expressed only cells of the defined lineage is highly unpredictable. Locations with nonsense sequence or comprising coding sequence for gene ubiquitously expressed or in regions would provide no information of the state of differentiation.

Alternatively, the vector comprising a promoter can be part of the genetic construct that is tissue or differentiation specific.

6) Amount of Experimentation Required. The invention recites use of a broad group of vectors and target sites to be used in homologous recombination. Given the unpredictability of the art, the poorly developed state of the art with regard to predicting the transformed cells absent a marker, the lack of adequate working examples and the lack of guidance for methods of identification absent a marker, the skilled artisan would have to have conducted undue, unpredictable experimentation to practice the claimed invention.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1-4, 7-10, 12 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Benvenisty et al (US 2002/0127715 or WO 02/061033); see entire documents) in view of .

Applicants claims are drawn to modification of hES cells by electroporation of a genetic construct comprising a foreign gene and homologous regions for recombination into the hES genome followed by identification of cells comprising the genetic construct and purification of the cells in which the marker is a marker of cell lineage of differentiation. Furthermore, gene expression profiled from the purified cells is analyzed.

Benvenisty et al teach electroporation of genetic constructs into human embryonic stem cells to transform cells with a construction comprising a marker for identification and purification of transformed cells(see e.g. ¶ 0008- ¶0009, 0044 and 0062). The construct can be a fluorescence marker or any other type of marker protein that is used to distinguish transformed cells from those absent sequence such as for example by FACS analysis (see e.g. ¶ 0008, ¶ 0010 and ¶0012). The marker comprises a promoter that is specifically active in cells in a desired state i.e. undifferentiated (see e.g. ¶ 0012 and 0047) or differentiated states leading to different lineages (see e.g. ¶ 0054).

Benvenisty et al do not teach that the vector is integrated by homologous recombination or that the marker is inserted into regulatory regions of the genome such that its expression is regulated according to its state of differentiation.

In ¶0180, West et al state that DNA markers can be inserted into human genes by homologous recombination. The markers are either inserted into sites so that they are transcriptionally regulated by the promoters of the genes into which they are inserted (see e.g. ¶0131) or comprise exogenous promoters that are development stage specific promoter/regulatory elements (see ¶0199). In these methods it is preferable to use homologous recombination for insertion of the construct comprising a marker into a specifically selected site in a gene that is conditionally expressed in a differentiating cell to disrupt and inhibit expression of the endogenous gene to produce a knockout or inserted to be transcribed ¶0073. The method of West et al allows for isolation of cells in distinct differentiated states such that the gene profile can be determined (see e.g. ¶0199).

It would have been obvious to one of ordinary skill in the art at the time the invention was made to methods of random insertion taught by Benvenisty with the methods of homologous recombination such as insertion of a promoterless marker into the genome in a sight that is regulated by the stage of differentiation as taught by West et al because Benvenisty et al teach that it is within the ordinary skill of the art to transform a hES by electroporation with markers to identify transformed and differentiated cells and because West et al teach that it is within the ordinary skill of the art to use homologous recombination with these cells for directed stable integration such as in specific regions or genes that are stage or lineage specific. One would have been motivated to do so in order to receive the expected benefit of insertion of the construct comprising a marker into a specifically selected site such that cells of specific lineage can be isolated and the genetic profiles of the cells determined. Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result in the claimed invention.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Maria B. Marvich, PhD whose telephone number is (571)-272-0774. The examiner can normally be reached on M-F (7:00-4:00).

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Joseph Woitach, PhD can be reached on (571)-272-0739. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Maria B Marvich, PhD
Examiner
Art Unit 1633